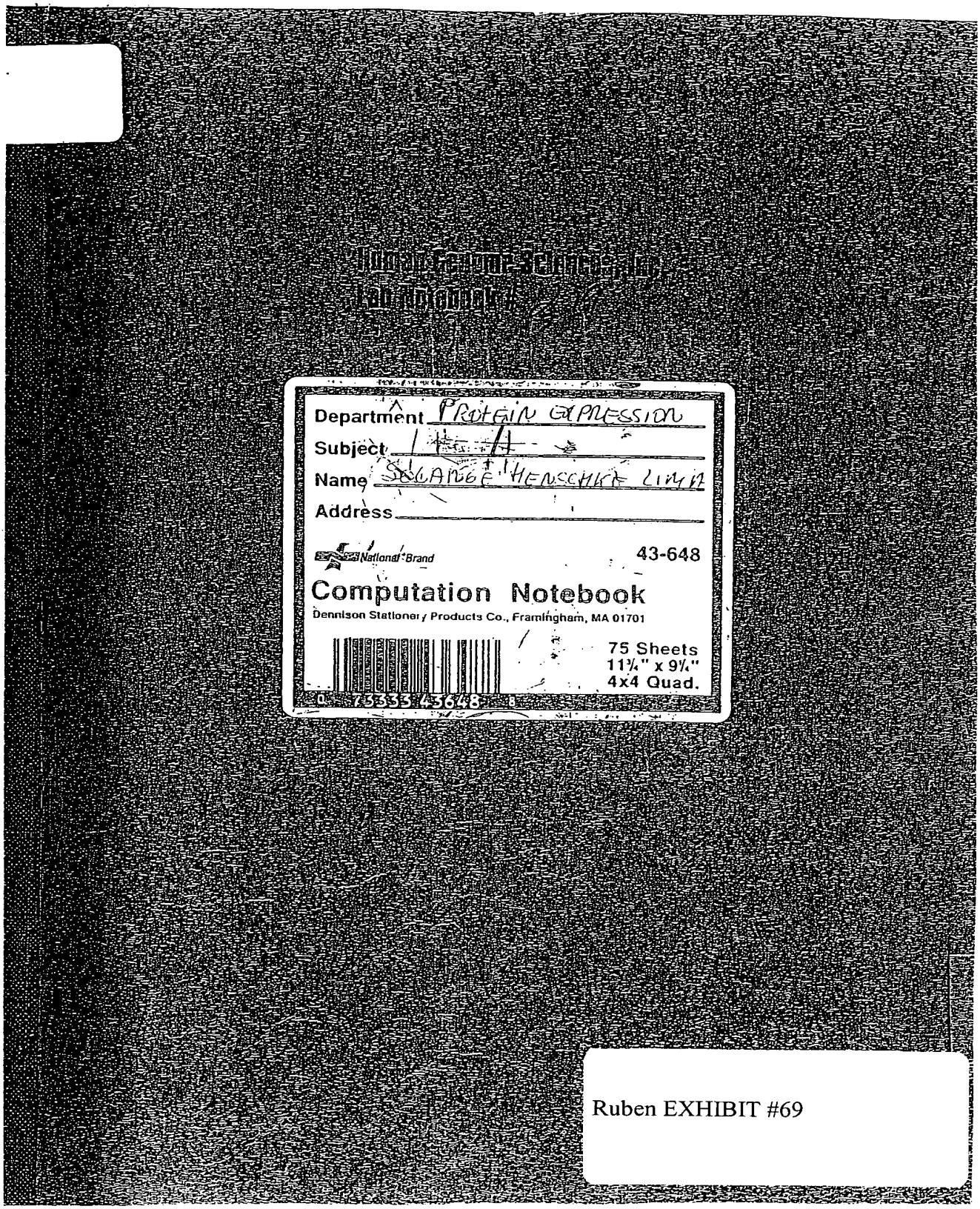


Ruben EXHIBIT #69

Department PROTEIN EXPRESSION

Subject LAB #1

Name SUZANNE HENSCHKE LIMA

Address

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

\#
1 A.e 30 μM → split to 60 μM Mtx
1 A.e 60 μM → " to 70 μM and MEMα+

\# 1 A.b 50 μM → split to 60 μM
1 A.b 60 μM → split to 70 μM Mtx and in MEMα+

\# 1 A.d 50 μM → split to 60 μM
1 A.d 60 μM → split to 70 μM Mtx and in MEMα+

2/17/95 Injections Spinner Flasks in EK-401 01-PBS

REDACTED

2/17/95 Transfections in Sf9 cells

REDACTED

3) HtPANO8SO4 - 51bp

4) HtPANO8SO4 - 185bp

The DNA concentration of all was ≈ 1 μg/μl

\#s 1, 2, 3, 4 I have the sequence report sheet

REDACTED

procedure like page 1

4

2/21/95 2 gave ~500 ml of supernatant to Mark

REDACTED

2/22/95 PLAQUE PURIFICATION:

REDACTED

HTPAN08S04-S1bp

HTPAN08S04-185bp

REDACTED

2 22 95.

REDACTED

2 22 95

2 22 95 2 harvested the transfections in Sf9 cells from 2.17.95

REDACTED

3) HTPAN08 SO4 - S1 bp

4) HTPAN08 SO4 - 1856 bp

REDACTED

REDACTED

3.1.95 Injection SJG cells with plaque purified virus of

REDACTED

HTPAN08504 - 51bp

* HTPAN08504 - 125bp

from 2.22.95

2 plaques of each

* 1 had plaques only in the first dilution

REDACTED

3.6.95 Harvested S19 cells infected on 3.1.95 with plaque purified lines
blue assay

REDACTED

HTPAN0 85 04 (-185bp) ① +++
HTPAN0 85 04 (-185bp) ② ++

HTPAN0 85 04 (-515bp) ① - (negative) ⇒ to be redone
HTPAN0 85 04 (-515bp) ② extremely weak
kept
D. obs: redone on 3.8.95

REDACTED

3/8/95 Injection of 9 cells with plaque purified virus of

REDACTED

3) HTPAV0850A-S16D from 2.2.95

REDACTED

3.12.95 Split CHO Stanniocalcin clones from 3.1.95

all 4 clones are finished and amplified up to 100 µM MTX
and the cells were seed today in MEMα+

StC ≠ 1A.a
StC ≠ 1A e
StC ≠ 1B.C
StC I 1A.b

3.13.95 Harvested transfections from 3.8.95

Dnase 04-101 → did not work for the 2nd time!!

HPFCT89X (P56-3) → blue assay positive!

3.13.95 Harvested Sf9 cells infected with plaque purified virus from 3.8.95

HIBCL22 (3) good
(4) weaker

HUVCT01S32 (VIGF) (1) both good
(2)

HTPAN08 - SIbp (5) did not work for the
(6) 2nd time → has to be plaque purified again

3.13.95 NOTE today Reiner send to Frank Kirschen 3 vials of FLAP, labelled as: V-HAPAK-1 = Flap 1 +16
V-HAPAK-2 = Flap 1 +14

18

3/14/95 Harvested the virus stock HTPAN0850 ÷ 185.bp
from 3.9.95 (not in the lab book)
the blue assay was positive

REDACTED

22

REDACTED

3 2375 Injections Spinner flasks in CX-01 PBS

REDACTED

1 4 HTPA ND8504:-18S b P (Fa, Ligand)

REDACTED

24

3 28 95 Harvested Spinners flasks injected on 3.23.95

REDACTED

1 11 HtPAnD8504 -185bp → Supe and cells to Nagase

REDACTED

REDACTED

REDACTED

3.30.95	Injections Spinner flasks in CX-401 of CB

REDACTED

2 specimens HtPAN08504 - 185bp

REDACTED

28

REDACTED

4/5/95 Harvested the spinner flasks inspected on 3/30/95

REDACTED

HIPAN08S04-1856P to Rajeev

REDACTED

REDACTED

9 11 95	PLAQUE PURIFICATION
	HtPAN08504 -S1bP Nohone

REDACTED

REDACTED

4.18.95 Injection Sf9 cells with plaque purified virus from 4.11.95

HTPAN08504 - S1bp was contaminated!

REDACTED see 4.21.95

I pick three plaques of each obs: the plaques were to small!!

SUPERVISOR- [signature]
DATE- 4/19/95

[signature]

REDACTED

4.21.95	Note: the Sf9 cells injected on 4.18.95 with plaque purified virus were not positive in the blue assay so I decided to purify them again
	⇓
4.21.95	PLAQUE PURIFICATION
	HTPAN08S04 - 36bp
	HFKEB40 6-4
	HD6RC10

REDACTED

4'21'95	Injection Lfg cells with plaque purified plaques from 4.21.95 HTPAN08504-S1 bp is small plaque

REDACTED

38

5 3 95 Harvested Sf9 cells injected with plaque purified virus from 4.27.95 blue assay according to Pam

HTPAM08504-S1, bP ① +

② +

REDACTED

5 5 15 Injection 293 cells for virus stock

HTPANOBSO4 -s1bp ⑤

REDACTED

Amount of virus: 50 ul/flask

REDACTED

HtPANOBSO4 -s1bp ②

REDACTED

blue assay
positive for all

REDACTED

5/19/95 thawed new Sf9 cells

1 vial of Sf9 in Grace 10% FBS - D.S. $\times 10^6$/ml P: 31-32

1 vial of Sf9 in ex-cell 21 FBS - D.S. 6×10^6/ml P: 31-32

Procedure:

- thaw the vials quickly in 37°C bath
- resuspend the cells and transfer them to a tube with 10 ml of media
- spin for 5' at 0.1 rpm
- discard the supernatant
- resuspend the pellet in 1 ml of media
- inoculate in 25 cm² t flask
- incubate at 27°C for 2 to 3 days

SUPERVISOR- [signature]

DATE- 5-19-95

72

6395 Harvested Spinners flasks injected on 9.29.93

2 spinners HT PBS 22 secreted proteins → David
2 " HMSBF 22 unknown → Rajeev
2 " Rat Stanniocalcin → Pedro
2 " PGF-1S HATCK89 → Rao
1 " HEHBE01-09 → Yibing
1 " Ea

10395 Transfections in CHO cells dhfr-

HUVEC91 → TNF δ pN346

HUVEC91 → TNF δ pCHO-1

see the Sequence Verification Report sheet for more information

TRANSFECTION, SELECTION, CLONING, AMPLIFICATION & SCALE UP of Recombinant CHO.dhfr- Cells.

PHASE I. PROCEDURE:

TRANSFECTION. Co-transfect CHO.dhfr- cells in 35mm ø culture dish using LIPOFECTIN.

PROCEDURE : (T1) Prepare the 35mm ø culture dish of cells the day before doing the transfection so that it is about 80% to 90% confluent.

(T2) Remove the culture medium and wash the plate once with PBS. Add 1ml. of OptiMEM, GIBCO Cat.No.041-01985M without FCS (or 1ml.of culture medium without FCS). Return plate to 37°C until required.

(T3) Prepare the following transfection mix in two separate POLYSTYRENE test tubes, NUNC Cat. No.3-41343A:-

A : 90µl OptiMEM or Culture medium without FBS. 10µl LIPOFECTIN. BRL Cat.No.8292SA.

B : 50µl OptiMEM or Culture medium without FBS. 5µl Plasmid DNA at 1µg per µl. 0·5 1µl Co-transfection DNA at 1 µg per µl. (G418 Resistance Plasmid pSV2 Neo).

Mix the contents of tube A with that of tube B and allow to stand for 10 to 15 minutes before addition to cells.

(T4). Remove culture plate from incubator and add the transfection mixture to it dropwise around the plate.

(T5). Return the plate to incubate at 37°C. Shake the plate forward and sideways in order to allow the DNA mixture to be evenly distributed over the cell surface. Incubate the plate for a further 4 to 6 hours After this, add 1ml. of medium containing 10%FBS and 200µg per ml.of Gentamycine. Return the plate to incubate for 24 to 48 hours before harvesting the cells for selection

10.4.95 Harvested CHO cells transfected on 10.3.95 and seeded them in selective media.

"d

PHASE 2

SELECTION OF RECOMBINANT CLONES

PROCEDURE : - (S1) Examine the transfected plate to make sure there is no microbial contamination. The cells should be confluent and in good condition.

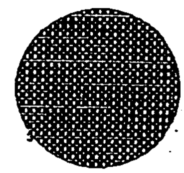
(S2) Remove the culture medium from the transfected plate and wash the monolayer with 2ml. of PBS. Remove the PBS and add 0.5ml.of Trypsin / EDTA GIBCO Cat.No.043-05300. and return to 37°C for 5 minutes.

(S3) Resuspend the detached cells in 5ml of selection medium :

Alpha(-)MEM (without Ribonucleosides & Deoxy ribonucleosides). GIBCO Cat.No.041-02561.
5% Dialysed FBS. GIBCO Cat.No.220-6300AJ.
800 to 1000µg per ml of G418 Sulphate (Geneticin). GIBCO Cat.No.066-1811.
0.01µM Methotrexate (MTX) (+ Amethopterin) SIGMA Cat.No.A-6770. (Prepare a 5 mM stock solution of MTX in Alpha(-)MEM. Sterilize through a 0.2µm. filter. Dispense in 5 ml. aliquots and store in the dark at -20°C).
100 to 200µg per ml of Gentamycine. Amimed Cat.No.4-07F00-H

Resuspend the cells well by gently sucking up and dispensing in order to have a homogeneous cell suspension.

(S4) Dilute this suspension to approximately 45 ml. Dispense 15 ml. of this suspension into each of three 'Hybridoma' cloning plates from GREINER Cat.No.633-160. Take care to fill all the micro-wells and to avoid air bubbles being trapped in them.



'Hybridoma' cloning plate.

(S5) Return the plates to incubate at 37°C for 10 to 14 days. The plates can be examined after a week but colonies are generally to small to be picked at this stage. There should be no need to change the medium over this period unless there are an unusually large number of colonies. If the medium needs to be replaced then use the same medium as above.

Lo the following procedure see on page 77

10.5.95 Injections Sf9 cells for virus stock

HETBE01 - E2 (endometrial tumor specific gene)

HETBE01 - D1 "

10.5.95 Liquidd today CHO Stanniocalcin 2 clones from 7.21.95 in roller bottles in MEM α + 50 nM MTX + 5% dFBS
obs: after passaging the cells two times in t-flask and did work well after 8 days.

74

10.6.95 Injections Spinners flasks in α-MEM C/FBS

Hannaocalcin	Stc-1-CS	2	spinner
HMSAF22	unknown	2	"
HTPBS22	excited protein	5	"
FGF-14		2	"
FGF-15		2	"

250 ul of radevirus/spinner

10.9.95 Harvested the 5ml cells Injected on 10.5.95 for virus Stock

HETBE01-D1 blue assay restriction for BstB

HETBE01-E2

10.10.95 Harvested Spinner flasks Injected on 10.6.95

Hannaocalcin	Stc-1-CS	1	spinner to Rajeev
HMSAF22	unknown	2	" to Rajeev
HTPBS22	excited protein	5	" to David
FGF-14		2	" to Padma
FGF-15		2	" to Rao

P. Pb. b

10.10.95 PLAQUE PURIFICATION

OIF Homolog pA2 HARAO44
" pA2gP HARAO44

from 9.28.95

Semaphorin homolog pA2 HMBE22
" pA2gP HMBE22

HT3SB90 A2 unknown from 7.25.95
Obs. this virus had to be plaque purified again because we finished by mistake with the 2ml stock plaque (1) that was good in S35 labelling!

obs: no good plaques after 6 days
redone on 10.17.95

10.12.95 Split the Stanniocalcin clones in CHO cells (roller bottles) from 10.5.95

I split the cells in MEM α - + 5% diFBS
+ 25 μM MTX
1:2 → 4 roller bottles after
150 ml of media / roller bottle splitting

10.13.95 Infection Spinner flasks in EX-401 w/o FBS

12 spinners HTPB622 secreted protein

250 ul of virus / spinner

[signature]

SUPERVISOR- [signature]
DATE- 10/13/95

10/13/95 PLAQUE PURIFICATION

Re-purification of viruses from 10.10.95 because I had no good plaques this time. I injected the Sf9 cells with dilutions from 10^{-4} to 10^{-8}

Unfortunately again I had no good results after 6 days of the purification and I will try from scratch and dilute the virus up to 10^{-12} or 10^{-13}

10/17/95 Harvested Spinners flasks injected on 10.13.95

HiPBS22 12 spinners
(ODGF)

I gave the supernatant to David

J. Ho

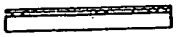
10.18.95 Picking clones of TNF & HIVEN91 CHO cells from 10.4.95

PHASE 1

CLONING

PROCEDURE

(C1) Remove the 'Hybridoma' cloning plates after 10 to 14 days of incubation at 37°C. Suck off the surplus medium with a pasture pipet. Then add 15 ml. of PBS to the dish and swirl around to wash the plate. Remove the excess wash WITHOUT removing any of the liquid from the micro wells.

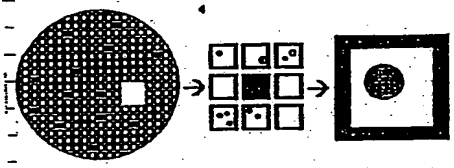


Inverted cloning plate. The surface tension prevents the liquid in the micro-wells from flowing out and prevents the plate from drying out.

(C2) Invert the plate (None of the liquid in the micro wells comes out). Observe the inverted cloning plate under a low power Binocular microscope (NIKON x10 to x30 magnification). This allows you to view a greater area of the plate and to chose colonies much faster.

we do not invert because we don't have this specific Nikon microscope. Instead, we look the clones in the normal position and we keep 5 ml of PBS while looking and labeling the clones with a pen.

(C3) With a bit of practice you should be able to pick out wells containing only one clone. Circle or color the well with a NUNC Cryo Pen Cat.No.319993. Choose 24 clones from different parts of the plate.



Most wells containing single clones *Well with a single clone*

(C4) Add 15 ml. of PBS to the plate for a final wash.

(C5) Method 1 : Suck off all the liquid (PBS) from the plate including the liquid in the micro wells.

Add 5 ml. of Trypsin/EDTA to the plate and swirl it around in order to fill all the micro-wells. Tilt the plate and suck off all the excess trypsin. (There is still enough trypsin held in each well to detach the cells). Incubate the plate at 37°C for 5 minutes.

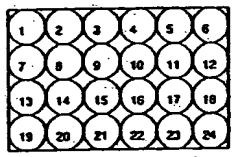
Method 2 : Only suck off the PBS from the micro wells which have been marked. Add 5 µl. of Trypsin/EDTA to the well and incubate at 37°C for 5 minutes. Remove the contents of the well and proceed as directed below in (6).

we do method 2

After removing the clones, add 15 ml. of PBS to the plate, swirl around and suck off all the liquid including that from the micro-wells.

Add 15 ml. of selection medium to the plate and return to incubator. This allows the plate to be used again to pick more clones if necessary.

(C6) Prepare three 24 multi-well plates with 1 ml. of selection medium (as described above but WITHOUT any G418) per well.



Number each wells in the three 24 multi-well plates from 1 to 72.

TAKE CARE TO TREAT EACH CLONE SEPARATELY IN ORDER TO AVOID CROSS CONTAMINATION BETWEEN CLONES.

Also: I pick 10 clones of the construct with pCMV-1 vector and 9 clones with the pV346 vector.

I had much more clones with the pCMV-1 vector.

10.1995 Split Roller bottles from 10.12.95 CHO Stanniocalcin & clones in MEM α^+ + 5% FBS

2 split 1 to 4 ⇒ total: 16 roller bottles

10.2095 Infections Spinner flasks

1 spinner HCFBF49 GP CHGF-3 in α-MEM 4%FBS

3 " FGF-15 HATCR89 "

1 spinner RAT Stanniocalcin ① + 1% H.I.FBS

1 " " ② + 1% H.I.FBS

10.2495 Harvested Spinner flasks infected above

2 sp. CHGF-3 → sup to Scott
3 " FGF-15 → " to Rao

The spinners of Stanniocalcin Pedro harvested on 10.25.95

10.2595 Split Roller bottles from 10.1995 CHO Stanniocalcin & clones in MEM α^+ + 5% FBS

9 split 1 to 4 ⇒ total: 64 roller bottles

Obs. 2 gave 4 L supernatant today to Alex for purification.

10.26.95 Amplification (Phase 4) of the CHO clones from 10.18.95 TNFζ HUVEOII

Procedure:

Look the cells and check if the clones are at least 60-80% confluent

Remove the media and make aliquots to check for the best expression

I gave to Dan Bednarik the circled ones

0FBS

100 ul of clone #s (1) (2) (3) (4) (5) (6) (7) 8 → I changed the media with the same MTX concentration as before = 10nM

9 10 → changed media

5

5

pCHO.1 →

pN346 → (a) (b) (d) (e) (f) (i) e → changed media g → " " h → died

wash the monolayer with 1 ml of PBS
Remove the PBS

Add 250 ul of trypsin-EDTA and incubate for 5' minutes at 37°C

Resuspend the cells adding to them 750 ul of media MEM α⁻ + 5% dialyzed FBS

Prepare the medica with the different concentrations of MTX that you decided to use

a. Dilute 10 µl of MTX (from 5 mM stock solution) in 1 ml of normal + S.I. dFBS

b. take this dilution (1 ml) and add it to 99 ml of medica and you will have the final concentration of 500 nM

Prepare the 6 well dish and add the amount of media with MTX and w/o MTX as below:

MTX concentration	500 nM ↓	normal media ↓
10 nM	40 µl	1.96 ml
30 nM	120 µl	1.88 ml
90 nM	360 µl	1.64 ml

For each clone you will have now 3 wells with 3 different concentrations (10, 30 and 90 nM) Add approximately the same amount of cells in each of the 3 wells (taking care not to mix the different clones)

Incubate at 37°C and look how the cells are growing in the next days

go to

10.27.95 Injections Spinners flasks in EX-40 WOFBS

8 spinners HMSAF22

4 " HtPBS22 (CODGF)

10 31 95 Harvested spinners flasks injected above

8 sp HMSAF22 to Rajan

4 " HtPBS22 (CODGF) to David

10 31 95 Harvested roller bottles (six) from 10.25.95

I gave the supernatant to Alex ≈ 13 l

and the media was replaced the same as before

10 31 95 PLAQUE PURIFICATION

Virus from 10.17.95 that did not work I diluted the virus from 10 to 10^{-3} and used the dilutions 10^{-5} + 10^{-13} in order to get better plaques

OIF Homolog pA2 HGCA044

" pA2gp HGCA044

Semaphorin homolog pA2 HMBE22

" pA2gp HMBE22

H#35B to

82

10395 Injections Spinner flasks in ex-401 4/0 PBS

12 spinners HAABR60 9-10
cystatin
250 ml of virus/spinner

10395 Harvested the supernatant from roller bottles (Stc 2) from 10.3.95

Flex spot ~ 12 l supernatant and Nam split 2 roller bottles 1 to 4 to keep as back up in MEM + 5% FBS

J Mo 10

PB.S 10.6.95 Amplification of CHO cells +NFL HUVECA

from 10.5.6.95

Procedure

Look how the cells are growing in the different mtx concentrations and if possible make a pool of cells growing good in the 2 highest concentrations (30 and 90 nM)

Remove the supernatant
wash the monolayer with PBS (1 ml)
Remove the PBS and add 250 ul of trypsin and incubate at 37°C for 5'
Add 250 ul of media and resuspend the cells

Prepare the media with the new concentrations of mtx for amplification

Media from 500 nM stock take 9 ml and add it to 41 ml of MEM α + 5% diFBS to have 90 nM and 20 ml of the stock and add it to 30 ml of MEM α + 5% di FBS to have 200 nM mtx

For each clone I have now 3 wells

- MEM α + 5% diFBS for Dan Bodnarik analysis
- 90 nM > amplification
- 200 nM

Clones in pCHO-1 vector

(1) 10 nM OK 30 and 90 cells died

(2) pool of 30 and 90 nM mtx

(3) " of 30 and 90 nM

(4) I used the cells growing in 30nM
90nM the cells died

(5) pool of 10 and 30nM.

(6) pool of 30 and 90nM

(7) pool of 30 and 90nM — clo. degrees 8 and 10 I discarded

(9) only 30nM I used

Clones in pN346 vector

(a) pool of 30 and 90nM

(b) only 30nM used

(d) only 90nM used — dies. Clones e and g I discarded

(f) pool of 10 and 30nM

(h) pool of 10 and 30nM

(i) pool of 10 and 30nM

do: I prepared 96 well for Dan Bednard in MEM α + 5% dFBS of each clone that I split today for assay

[initials]

10.7.95 Harvested spinner flasks injected on 10.3.95

12 spinners of Captation -> super vialing
HAG BR 60 9.10

10.8.95 transfection in Sf9 cells

HCARA 58 X (extracellular protein (EGF domain))
plasmid concentration 15 ug/ul
procedure like page 1

10.8.95 Infections Sf9 cells with plaque purified virus from 10.31.95.

OIF Homolog pA2 HARAO44
OIF " pA2gp HARAO44

Semaphorin homolog pA2 HMBE22
" " pA2gp HMBE22

H735B70 unknown

3 plaques of each but the plaques were too small

11.10.95 Infections Spinners flasks

8 spinners in EX-401 of cystatin HAQBM60.9.10
↳ harvested on 11.13.95

2 spinners for testing new media 15 Bac from Irvine Scientific (w/o FBS)

A2 Regeneration protein HCQB17
ODEF HTPBS22

2 spinners of A2 reg and ODGF in EX-401 w/o FBS to compare how good is the new media 15 Bac (see the gel next page)

11.10.95 obs: I gave today to Jan Bednarik the 96 well plate and supernatant from clones of HOVED91 in CHO cells for testing antiviral activity. Obs the cells were prepared 11.6.95

11.13.95 Harvested Sf9 cells infected with plaque purified virus from 11.8.95

OIF Homolog PA2 HARAD44	(1) ++		Semaphorin homolog HMBE22 PA2	(1)	++
	(2) ++			(2)	+
	(3) ++			(3)	
OIF homolog PA2SP HARAD44	(1) +++		Semaphorin homolog HMBE22PA2SP	(1)	++
	(2) ++			(2)	++
	(3) ++			(3)	++
			HI35S72 unknown	(4)(5)(6)	++ ++ ++

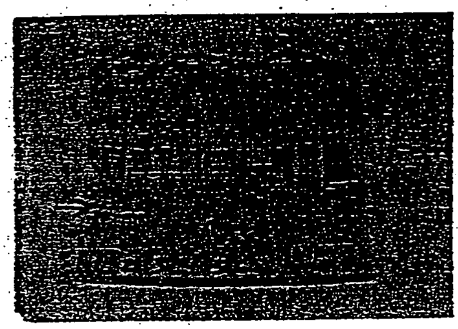
13B95 Transient transfection from 11B93

11CAB3A55x (extracellular protein/EGF domain)

blue assay + but not so strong

11B95

Western ... 11B93



1, 2 Expression OK ... 101:139

3 CDEF (EY-4..) we can see the band below 55

4 CDEF (15 BAC) we can not see the band below 55

5 A_2 Regulation protein (EY-4..) we can see the band

6 A_2 Regulation protein (15 Bac) we can see the band

Conclusion: the 15 Bac media seems to be OK but as we can see CDEF was not secreted so we better try to adapt the cells in spinner before infection to make our final conclusion!

J. M. B.

11/5/95 Amplification of CHO cells TNF + HUVEC91 from 10 6 95

Clones in pCMo.1 vector

#	2-	90nM	200nM	comments
1	OK	Died	Died	split 2 cells in 30nM MTX
2	OK	few clones	Died	split some cells again in 90nM
3	OK	very good	good	pool of 90 and 200 in 200nM
4	OK	few clones fighting	Died	split 90nM in 90nM
5	OK	few clones fighting	Died	split 90nM in 90nM
6	OK	very good	very good	pool of 90 and 200 in 200nM
7	OK	few clones	Died	split 90nM in 90nM
9	OK	few clones	Died	split 90nM in 90nM

Clones in pN346 vector

#	2-	90nM	200nM	comments
a	OK	good	few clones fighting	pool of 90 and 200 in 100nM
b	OK	few clones	" " "	pool of 90 and 200 in 100nM
d	OK	good	few clones	
e	OK	clones fighting	some cells	
f	OK	few clones	died	pool of 90nM in 100nM MTX
h	OK	good	some cells	pool of 90 and 200 in 100nM

the media was prepared like page 83 and the procedure for the cells also
According to Dr. [illegible] the clones #s 3, 5, 7 and b, e showed some antiviral activity

11/15/95 PLAQUE PURIFICATION

HCABA58X (extracellular protein / EGF domain)

from 11 13 95

11.16.95 Injections Spinners flasks in EX-401 w/o FBS

11 spinners of Cystatin HAQBM60 9-10

11.20.95 Harvested spinners injected above

Supernatant to Yuling

11.21.95 Amplification of CHO cells IFN-γ [illegible] from 10.15.95

Clones in pcDNA vector

#	MTX Concentration	Comments
3	100 nM MTX	excellent, confluent > pool 200, 300 nM MTX
3	200 nM	″ ″
6	100 nM	very good ″ > pool 200, 300 nM
6	200 nM	″ ″
1	30 nM	good → split 50 nM and 100 nM
2	90 nM	good → ″ 150 nM and 100 nM
4	90 nM	few clones fighting > change media to 100 nM
	90 nM	
5	90 nM	some cells fighting > ″ ″ 100 nM
	90 nM	
9	90 nM	some clone → ″ ″ 100 nM
9	90 nM	″ → ″ 100 nM

Clones in [illegible] vector ⟹

pN346 vector

mtx

#	Concentration	Comments
a	100 nM 100 nM	v.good 90% confluent > pool 100,200 v.good " > and seed in flask
b	100 nM 100 nM	few clones > change media to 100 nM "
d	100 nM 100 nM	excellent > pool 100,200 + flask "
e	100 nM 100 nM	few clones > change media fighting > to 100 nM
f	100 nM 100 nM	" > change media to 100 nM
i	100 nM 100 nM	few clones > change media " > to 100 nM

Media preparation:

From 500 nM Stock to prepare:

100 nM 5 ml of the stock into 20 ml of MEM α-
200 nM 10 ml " " 15 ml "
300 nM 15 ml " " 10 ml "
50 nM 0.2 ml into 1.8 ml "
150 nM 0.6 ml " 1.4 ml

[signature]

11.20.95 Infections Spinners flasks in ex-401 (11/17/95)

8 spinners HMSAF22

2 " 6P-F spondin HESA920

2 " A2-F spondin HESA920

amount of virus 180 ul / spinner
due to the Holidays we used less this time!

11.22.95 Infections Sf9 cells with plaque purified virus from 11-15-95

HCABA58X (extracellular protein / EGF domain)
3 plaques

11.27.95 Harvested Sf9 cells injected above

		blot assay
HCABA58X	(1)	+++
"	(2)	++
"	(3)	+

11.27.95 Harvested Spinner flasks injected on 11.22.95

8 spinners HMSAF22 → sup to Stee

2 " 6P-F spondin } HESA920
2 " A2-F spondin } "

sup and cells
[illegible]

11/18/95 Amplification of CHO cells +MF2 #UV091 from 11.21.95

Clones in pCHO-1 vector

#	MTX concentration	comments
3	200 nM	Very good and confluent
3	300 nM	split in 300, 400 nM MTX → bigger flask
6	200 nM	good, 80% confluent cells
6	300 nM	floating a little bit, split to 300, 400
1	d-	OK but I won't use it anymore
1	50 nM	many clones, split to 50 nM in t flask
2	d- [illegible]	OK but I won't use it anymore
2	50 nM	cells look OK; confluent split to 50 nM to t flask
4	100 nM	good clones, make a pool
4	100 nM	split to 100, 150 nM in t flask
5	100 nM	very good clones, make a pool and split to 100 nM t flask
9	100 nM	overconfluent split to 100 and 150 nM t flask
9	100 nM	→ 80% confluent, split to 100 and 150 in t flask

Media preparation from 500 nM MTX stock

		MEM 1-
50 nM	12.5 ml	22.5 ml
100 nM	10 ml	40 ml
150 nM	15 ml	35 ml
200 nM	20 ml	30 ml
300 nM	30 ml	20 ml
400 nM	40 ml	10 ml

Clones in pN346 vector

#	MTX Concentration	Comment
a	100 nM	good split to bigger
a	200 nM	very good flask, 200, 300 nM
d	100 nM	confluent but the cells have
d	200 nM	a different shape. Pool and split in bigger 2 sp flask
b	100	no clones make a pool
b	100	and split in 100, 200 (+flask)
e	100	good clone, the same
e	100	like b (100, 200)
f	100	few good clones, pool
f	100	and split in [illegible] -flask
λ	100	good clones the same
λ	100	like b (100, 200)

11/28/95 Injection of 9 cells for virus Stock

HMQBE22 (1)A2gp Semaphorin homolog from 11.3.95
obs: this clone is not full length

obs: HARAO44 PA2 -> OIF Homolog
" PA2gp was not expressed (S35 labeling)

HMQBE22 A2 was expressed but not secreted
and HMQBE22 A2gp was secreted [illegible]

11/28/95

H135B?C plaques 4,5,6 we found no expression

11 30 95 Infections spinners flasks (ex-401 w/o FBS)

12 spinners A2HNGF HHPSH35

250 ul of virus / spinner

12 1 95 transfections in Sf9 cells

pA2 HSAAU35 (X-CSFR) 0.5ug/ul DNA: mini-prep!!!

pA2 HBJEL88 (thymus specific unknown) 1 ug/ul

procedure see page 1

12 4 95 Harvested Sf9 infected on 11.28.95 for virus stock

HMEBE22(1) pA2GP semaphorin homolog

blue assay positive

12 4 95 Freezing of TNF clones (HUVE091) in CHO cells from 11.28.95

Freezing media: 5% DMSO, 10% FBS MEM α+

clones #: 3 300 nM MTX > cells looked very good
3 400 nM MTX > pCHO-1 vector

6 300 nM MTX > 90% confluent cells looked
6 400 nM MTX > ok pCHO-1 vector

d 150 nM 85% confluent, cells looked ok
pN346 vector

a 200 nM
a 300 nM } cells looked good
pN346 vector

I prepared 3 vials with 1 ml of each clone and the cells are resistant up to the concentration of MTX indicated.

12.5.95 Harvested Spinner flasks injected on 11.30.95.

12 spinners A, TNF HXPS35
Supernatant to Scott

12.5.95 Split TNF clones (hUVEC91) CHO cells from 11.28.95 in MEM α+ for freezing next week

pCho-1

#	MTX growing concentration	
1	50 nM	→ many nice clones
2	50	→ many single cells (??)
4	100	→ many clones starting
4	150	→ " " "
5	100	→ few good clones
7	100	→ nice cells 50% confluent
7	150	→ cells growing good
9	100	→ 95% confluent - good cells
9	150	→ 50% " "

pN346 vector

#	growing MTX concentrations
e	100 nM very good cells 85% confluent
e	200 very good clones 60% "
d	100 nM 90% confluent, nice cells
i	100 nM many single cells (??)
i	200 nM 100% confluent, different shape
b	100 nM excellent 95% confluent
b	200 nM nice clones 65% confluent

12/6/95 Harvested transfection in Sf9 cells from 12/1/95

HB3EL88 thymus specific unknown
rice array was positive

12/6/95 PLAQUE PURIFICATION

HB3EL88 thymus specific unknown

procedure see page I

12/7/95 Infections spinners flasks in Ex-401 w/o FBS

4 spinners A2 VNK 36 A.1 HCEDD36A

4 " GP-F spondin HESA970

4 " GP 16 f 2 Link #1 HTECD31

12.7.95 Harvested transfection in Sf9 cells from 12.7.95

obs: Blue assay was weak

HSAAU35 (X-CSFR)

12.8.95 Freezing of +NF clones (HUVEO91) in CHO cells from 1.5.95

obs: all clones frozen today were good and confluent and I made 3 vials of each

freezing media: 10% FBS, 5% DMSO in MEM α

pCHO-1 vector:

1 amplified up to 50nM MTX
9 100 nM
9 150 nM
7 100 nM

pN346 vector:

d 100 nM
b 100 nM
b 200 nM
a 200 nM
e 100 nM

12.11.95 Harvested spinners flasks infected on 12.7.95

4 spinners A2 UNK 36 A-1 HCEDD36A supe to Stephen
4 " GP-F spondin HESA320 supe to Pedro
4 " GP EGF d Like 1 HTEC031 " to David

12.12.95 Freezing of TNP clones (Nuvear) in CHO cells from 12.5.95

As all clones (tho ext) frozen today in general looked good but some were less confluent than others that's why I prepared 3 or 2 vials of each.

pN346 vector

2 amplified up to 200 nM MTX 3 vials

1 " 100 nM 2 vials

p CHO-1 vector

4	100 nM	3 vials
4	150 nM	3 vials
2	50 nM	3 vials
5	100 nM	2 vials
7	150 nM	3 vials

12.14.95 Infection Sf9 cells with plaque purified virus from 12.6.95

HB4CL88 thymus specific gene
3 plaques

12.14.95 Infection Sf9 cells for Virus Stock from 11.28.95

HCABIA58 (3) extracellular protein EGF domain

12.14.95 PLAQUE PURIFICATION
from 12.7.95

HSAAU35 (X-CSFR)

procedure: like page 1

see 12.19.95

12.14.95 Infections Spinner flasks in EX-400 1% FBS

HTECD31 TGF2 like gp 6 spinners

HCABA58(3) extracellular protein ELF domain
2 spinners

gp HMQBM23 4 spinners

250 ul of viral spinner
Harvested by Nam on 12.18.95

12.19.95 Harvested (by Nam) Sf9 cells infected on 12.14.95
with plaque purified virus

HB9EL88 thymus specific gene (1)
(2)
(3)

12.19.95 Harvested virus stock infected on 12.14.95
who Nam prepared the stock

HCABA58 (3) extracellular protein ELF domain

SUPERVISOR: [signature]

DATE: 12/19/95

12.19.95 PLAQUE PURIFICATION (redone by Nam and he infected with the Sf9 cells with the plaques!

HSAAU35 (X-CSFR)

1996

1396 Injections Spinner flasks in LX-400 w/o FBS

1 2 spinners: HCABA58 (Extracellular protein Elaf domain)

2 " TGF 2 active domain (HTEC031)

5 " HNEC (HHPSH35)

5 2396 Thawing of CHC clones of TNF (HUVE091) in pCMO-1 vector

clone numbers	concentration of MTX already resistant:	thawdate:
4	100 nM	12.12.95
4	150 nM	12.12.95
2	50 nM	12.12.95
5	100 nM	12.12.95
1	50 nM	12.8.95
9	100 nM	12.8.95
9	150 nM	12.8.95
3	100 nM	12.8.95
3	300 nM	12.4.95
3	400 nM	12.4.95
6	300 nM	12.4.95
6	400 nM	12.4.95

All cells were seeded in MEM1+ + 5% FBS

1 2 96 thawing new crochip D944

Concentration / vial : 1 x10^6 cells / ml
12.20.95 - 4
frozen by Jinli

the cells were seeded in MEMα + 5% FBS

1 23 96 Infections Sf9 cells for virus Stock

HBJEL88 (2) thymus specific - pA2
50 ul of virus / T225 + flask

1 23 96 Harvested Spinners flasks infected on
1.18.96

2 spinners HCABA58 → Stephen
2 " TGFα active domain → David
5 " HNbF → Scott

1 23 96 Transfections in Sf9 cells

HTOJK64 (OAP AC) pA2 0.39 ug/ul (Ping Feng / P. Dillon)

HETG170 (ESBF II) pA2 0.6 ug/ul (Guo-Liang Yu / Jian Ni)

HFCB502 (Cystatin II) pA2GP 1 ug/ul (Guo-Liang Yu / Jian Ni)

procedure like page 1

102

1.24.96 Amplification of TNF (HUVEC-S!) in CNO cells

thawed on 1.22.96

clone #	amplified up to	split to
9	100 mM MTX	200 nM MTX
9	150	300
7	150	300 nM
1	50	100 nM
4	100	200 nM
4	150	300 nM

Media: from 500 nM MTX stock } dilutions:
100 nM: 2 ml of the stock + 8 ml of α- } 10 µl + 1 ml = 1:100
200 nM: 20 ml " 30 ml of α-
300 nM: 30 ml " 20 ml of α-
= 500 nM

In general all cells looked good and had 70 to 95% confluency.

The other clones #s 2, 3 and 6 probably will be amplified tomorrow 1.25.96

	clone #	amplified up to	split to
1.25.96	2	50 nM	100 nM
	3	400	500
	3	300	400
	6	300	400
	6	400	500
	7	100	200

1 26 96 Injections Spinners flasks in EX-401 W/o FBS
obs. The cells were prepared the day before

3 spinners HB9EL88 Thymus specific
2 " HHWAF38 EMAP II F. 2
2 " HTPBS22 ODGF OAP + H. H.I.F.B.S
3 " HRDCD54 Myelin oligod.
2 " HSAAU35 CSF-R like
400 ul of Virus/spinner — obs. Harvested on 1 31 96

1 26 96 Transfections in CHO dhfr- D944

HTPBS22 (appC102) ODGF pC-1 vector
DNA ≈ 2.5 ug/ul — Pingfeng/Patricia

HWFBD68 CKB-1 pC-1 vector
DNA ≈ 0.6 ug/ul — Haodong Li

procedure see page 72

1 26 96 Harvested Sf9 cells infected
on 1 23 96 for virus stock

HB9EL88 (2) thymus specific
pA2 — A2 Thymus spec #2 HB9EL88 HC12580 1/26/96
blue assay ++

1 2996 Selection of Recombinant clones (CHO dhfr-B944) transfected on 1.26.96

HTPBS.22 (appC102) pC-1 vector
HWEBD628 (CKB-3) pC-1 vector

procedure and media preparation see page 73

1 2996 Harvested transfections in Erg. from 1.23.96

		blue assay
HTO3K64	(OAP Δe) pA2	+++
HET6170	(ESBF II) pA2	+++
HFCB502	(Epiplatin II) pA2.6P	+++

1 2996 Amplification of TNF CHO clones (HUVEC691) from 1.24.96

clone #	amplified up to	split to	
5	200nM MTX	300nM MTX	clone (lighting 488)
9	300	400	
3	100	200	
9	200	300	
9	300	400	
4	200	300	
4	300	400	

clones from p. 61

0944) 1.30.96 Amplification of tNF CHO clones (HUVE091)
from 1.25.96

Clone #	[MTX]	split to
2	100 nM MTX	200 nM MTX
3	500	600
3	400	500
4	200	300
6	400	500
6	500	600

the clones are growing pretty good.

2.1.96 PLAQUE PURIFICATION (did not work)

HTOJK64 (DAP ΔC) pA2

HETG170 (ESBP II) pA2 — diluted from 10^{-1} to 10^{-10}

HFCBS02 (cystatin II) pA2 GP

HOUCK17 Muth-1 A2 } from Tim
HOUCK17 Muth-1 GP }

procedure see page 1

2.1.96 Amplification of tNF CHO clone (HUVE091)
from 1.30.96

	split to
# 3 (500)	600 nM MTX
# 3 (600)	700 nM MTX

this clone is growing fa[illegible]

SUPERVISOR [signature]
DATE 2/1/96

2 2 96 Injections Spinners flasks in CX-401 w/ PBT

1 spinner HTABX03 (EMAPII)
6 spinners HB.9EL88 (thymus specific)
5 " 1G1F

300 ul of virus/spinner

2 5 96 Amplification of CHO clones TNF (HUVEC91)
from 1.29 and 1.30.96

Clone #	MTX resistance	split to:
3	700	Kept
3	600	Kept
2	200 good, confluent	300 nM MTX
2	300 " 70% "	400
6	600 " 80% "	700
6	500 " 80% "	600
5	300 few clones	300
1	200 good, confluent	300
7	400 " "	500
9	400 " "	500
9	300 ok 80%	400
4	400 ok 70%	500
4	300 good, 80% conf	400

media: from 1000 nM MTX stock

300 nM → 6 ml → 14 ml α + di FBS
400 → 8 ml → 12 ml
500 → 10 ml → 10 ml
600 → 3 ml → 2 ml
700 → 3.5 ml → 1.5 ml

2.6.96 Harvested spinners flasks injected on 2.2.96

1 sp HTABK03 (EMAP III) supe to Jeffrey
6 sp HB9EZ88 supe to Yuling
5 sp A61F supe to Pedro

12.7.96 PLAQUE PURIFICATION (repitition from 2.F96)

HT09K64 (DAP) pA2 supde De
HEt6170 (ESBF II) pA2
HFCBS02 (Cyptatin II) A2gP
HOUCK17 Meth-1 A2 } from Tim
HOUCK17 Meth-1 gp

I made dilutions from 10^{-1} to 10^{-13}

Procedure like page I

2.7.96 Transfection in Sf9 cells

HTOAU65 TNFR (soluble)

pA2 gp Project code H602800

Construct from Jian Ni

DNA concentration: 0.4 ug/ul

2.9.96 Injections Spinner flasks in EX-401 w/o FBS

5 spinners HRBC054
2 spinners HtABK03
4 spinners HBJEC88
1 spinner HBJEC88 + 1% HI.FBS

amount of virus/spinner: 400 ul

2.9.96 Amplification of TNF clones in CHO cells from 2.5.96

clone #

1(300) very good split to 400nM MTX
5(400) not good, few cells growing - Keep
7(500) good split to 600nM MTX
9(500) good " to 600nM
9(400) Very good " to 500nM
9(500) very good " to 600nM
4(400) very good " to 500nM
3(600) good " to 700nM } made a pool
3(700) " " to 700nM } regrown I had less cells
2(300) Very good " to 400nM
6(700) " " to 800nM
6(600) " " to 700nM
7(400) few cells growing - Keep

Media from 1000nM stock

600nM -> 9ml + 6ml mem α + 5% dFBS
500nM -> 5ml + 5ml
400nM -> 4ml + 6ml
700nM -> 7ml + 3ml
800nM -> 4ml + 1ml

2 13 96 Harvested spinners flasks injected on 2956

5 sp. HRDCD54 sup to Arvind
2 sp. HABK03 sup and cells to Jeff
4 sp. HB9AL88 → supe to Yuling
1 sp HB9AL8 + PBS

2 14 96 Harvested transfection in Sf9 cells from 2 7 96

HTOAU65 TNF R (soluble)

pA2 GP Project code: HG02260

2 14 96 Picking clones from CHO cells dhfr- D944 in selective media from 1 29 96

HTPS22 (sop C102) PC-1 vector
HWEBD68 (CKB-1) PC-1 vector

we have many clones of each construct this time!

Procedure was a little bit changed this time:

take the hybridoma plate, discard the media and wash twice with PBS
Remove the PBS and add 5ml of trypsin
incubate at 37°C for 5 minutes
with your scope inside the hood examine your best clones and with a bit of practice pick one by one and seed in MEM2 + 5% FBS (if you want to test for antiviral activity) or in selective media + MTX (20nM) + PS
→ MEM2 + 5% dFBS

In both cases we seeded this time in 1 clone
mono d*. (24 well dish) 1 ml of media/well

- Incubate the cells at 37ºC and look in 3 or 4 days how the clones are growing.

I pick this time 48 clones of OAP
48 clones of CKB-1

and as a back up I changed the
on 2.15.96 a media of 1 hybridoma plate of each gene and incubated 37ºC for a week also.
I used 20nM MTX instead of 10nM

2.13.96 Amplification of tNF clones in CHO cells from 2.9.96

Clone: 1

	3 (700)	60% confluent, fighting a bit, split to 800nM MTX
	2 (400)	confluent, very good, split to 500nM
	6 (400)	> 50% confluent but fighting for this resistance,
	6 (800)	split to the same concentration of MTX
media	5 (400)	cells died
from 100mM		
50% small 1 same	7 (400)	40% confluent, cells look ok, split to 500nM
600: 6ml 1 4ml	1 (400)	V. good: " to 500nM
900: 14ml 16ml	9 (600)	OK split to 800nM
800: 8ml 12ml	9 (500)	> very good split to 600 and 800nM
	9 (600)	
	4 (500)	V good -> split to 600nM
	4 (400)	V. good " 700nM

2.16.96 Injections Spinners flasks in Ex-401 cells 5

2 spinners thymus specific HBSEL88
5 " A2 46F2 full length HTECD31 (8-)
5 " HRDCA54.

2.16.96 Injections Sf9 cells with plaque purified virus from 2.7.96

HTO9G64 (OAP) pA2
HETEB70 (ESBF II) pA2
HFCBS02 (Cystatin II) AsgP } from tim
HOUCK17 Meth-1 A2
HOUCK17 Meth-1 A2 Sp

2.16.96 Injection Sf9 cells for virus stock
HSAAU35 (X-CSER) #3
50 ul of virus / # flask

2.20.96 Harvested Spinners flasks injected on 2.16.96

2 sp HBSEL88 → to Yuling
5 sp A2 46F2 → to David
5 sp HRDC A54 → to Arvind

ds: this was the day of our moving to 9410

2.21.96 Harvested Sf9 cells infected on 2.16.96 for virus stock

HSABU35 (GCSF-R) #3 — A2 GM-CSF Rec #3 HSABU35 ... 2/21/96

blue assay was good.

2.21.96 Harvested Sf9 cells infected on 2.16.96 with plaque purified virus

HFO9K64 OAP pA2

HETGI70 ESBPII pA2

HFCBS02 Cyptichin II A gp

HOUCK17 Meth-1 A2 } for tim

HOUCK17 Meth-1 A96P

blue assay was good for all

2.22.96 Changed the media of CKB-1 clone #HWFBD68 and HFPBS22 (zip clo2) OAP in CHO cells from 2.14.96 and TNF clones from 2.13.96

obs: I used MEM α^+ +5% FBS in order to give samples for assays.

HFPBS22 - OAP → I gave to Dan Bednaux
clones #s 1 to 48
49 CHO dhfr- 7944 supernatant
50 MEM α^{+} + 5% di FBS

HWFBD68 CKB-1 I gave to Erik for the Biacore
clones #s 1 to 48
49 CHO 7944 supernatant

Results: for the antiviral assay Dan did not detect antiviral activity

for the Biacore assay see the results on 2.29.96

2.23.96 Amplification of CHO clones: OAP, CKB-1, TNF

1) OAP #+PB522 - split all 48 clones in 20 nM mtx

2) CKB-1 #WFBD68 - split all 48 clones in 20 nM mtx

3) TNF clones #LVE091

clone #

4(600) split to 700 nM mtx
4(700) " 800

9(700) " 800 all clones looked
9(600) " 700 in general good!

7(500) " 600 media prep
7(700) " 800 for 1000 nM mtx
600 nM: 9 ml + 6 ml w/ mtx
1(500) " 600 700 nM: 7 ml + 3 ml
800 nM: 10 ml + 4 ml
2(500) " 600 900 nM: 9 ml + 1 ml
3(800) " 900

6(700) " 800
6(800) " 900

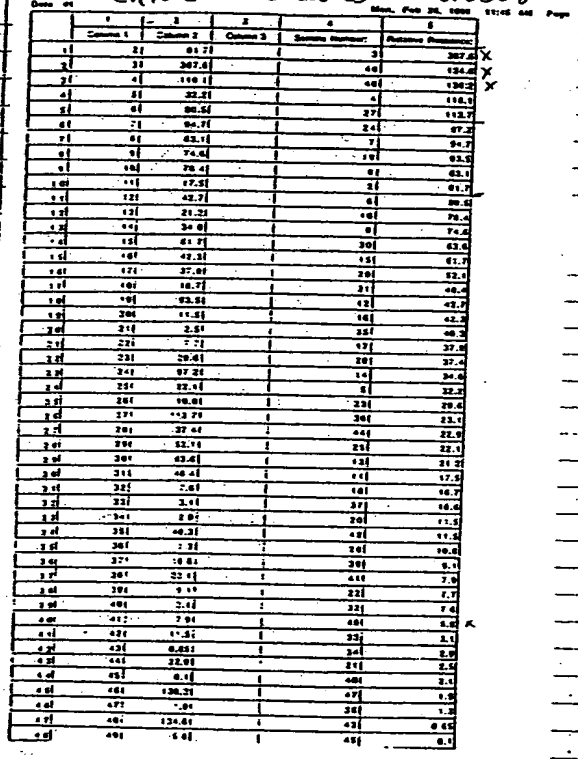
968

114

22796 Amplification of CHO clones: OAP, CKB1 from 22396

CKB1: Results from the Biacore

CKB1 - CHO clones HWFB968



clones that we pick to amplify:

ts 3, 48, 46 seeded in 20, 30 and 50nM MTX

clones that we pick to keep as back up:

ts 4, 27, 24 seeded in 20 and 40nM MTX

OAP: HTPB522

split all clones 1M 40nM** for amplification and 20nM** for SDS labelling on 3.1.96

2.27.96 thawed new Sf9 cells

1 Vial P.31-32 seeded in Grace 10% H1FBS
1 Vial P.31-32 " in EX-401 2% FBS

2.28.96 transfections in Sf9 cells

HHPEW62 from Yi Li pA2 DNA ≈ [0.5 ug/ul]
HSUA186506 from John Greene 16F-1 mature peptide fused to gp signal peptide - DNA ≈ [1 ug/ul]

Procedure like page 2

2.28.96 Amplification of TNF-human CHO clones from 2.23.96

clone #s

4 (700)	split to	800 nM MTX	
4 (800)		900	
9 (700)		800	
9 (800)		900	
2 (600)		700	all clones looked
1 (600)		700	OK
7 (600)		700	more than 80% confluency
7 (800)		900	
3 (900)		1000	
6 (800)		900	these clones the cells
6 (900)		1000	looked a bit bit fighting for the resistance

from 1000 uM MTX stock

Media:

800 nM	→ 8 ml	→ 2 ml
900 nM	→ 18 ml	→ 2 ml
700 nM	→ 10.5 ml	→ 4.5 ml

SUPERVISOR- [signature]
DATE- 2/29/96

3.1.96 Injections spinner flasks in EX401 WIC FS1

4 spinners VEGF-3 HOSBDY7 0400
4 " VEGF-3 HMWCFO6 0460
2 " A35

1 " HOUCK17 Muth-1 (3) Az
1 " HOUCK17 Muth-1 (3) GP

400 ul of virus/spinner

3.1.96 S^{35} Labelling of DAP. CHO clones from 2.27.96

obs: Reiner labelled the cells for me

The cells were kept in the same media 2mM MTX, 5% dFBS in MEM α- and the radioactive lys, (2 ul/well) was added.

100ul of the lys was diluted in 900 ul of the media and the amount of radioactive material/well was ≈ 20 uCi.

3.4.96 Kathy prepared the gel and loaded the samples for exclusive.

3.4.96 Amplification of CKβΔ clones from 2.27.96 and CAP clones.

CKB1

clone #s (for amplification)

3
46
48

I discarded the 20nM and seeded the cells growing in 30 nM in 40nM and the cells growing in 50nM in 60nM

Clone #s (for freezing)

4
24
27

I discarded the cells growing in 20 nM of #4 and #27 and seeded the cells growing in 40 nM in 50nM.

#24 I made a pool of 20 an 40 nM and split in 50 nM because I had less cells

All clones looked in general good!

OAP: htPBS22 (48 clones)

The cells of clone # 20 died and was discarded

All the rest I split in 60nM MTX and in general all clones looked good!

3.5.96 Harvested Spinners injected on 3.1.96

the supe of VEGF-2, VEGF-3 and A35 I gave to Scott

the supe of Meth.1 (3) I gave to Alex

3.5.96 Harvested the transfections from 2.28.96

Obs: HSVA 386 506 gp was contaminated! probably is the DNA! ↳ to be redone ↳ on 3.4.96

HHPEN62 was not contaminated but the blue assay was a little bit weak but positive!

3.6.96 Amplification of TNF clones from 2.28.96 ↳ HUVEO91

clone #s

7 (700) discarded

7 (900nM) split in MEM d- + 5% dFBS, 1, 3 and 5 μM MTX

3 (1000nM) " in " ; 2, 4 and 6 μM

6 (900) discarded

6 (1000nM) split in MEM d-, 2, 4 and 6 μM

1 (700nM) " in " ; 800nM, 1 and 2 μM

7 (700 nM) " in " , " , 1 and 2 μM

9 (800) discarded

9 (900) split in MEM2⁻, 1, 3 and 5 μM

4 (800) discarded

4 (900) split in MEM2⁻, 1, 3 and 5 μM

96

3.6.96 PLAQUE PURIFICATION

A, HHPEN62 (from 3.5.96)

HTOAU65 gp TNF R soluble (from 2.14.96)

A2 IL-10 homol. HMQBM23 → from ham

GP IL-10 " HMQBM23

3.8.96 Injections Spinners flasks in cells work

2 spinners 16F2 HTECD31
6 " HCEDD36 FSP-2

3.8.96 Transfection in Sf9 cells

HSVA 186 SOG gP from John Greene
= IGF-I mature peptide fused to gp signal peptide

Redone for the second time
see 3.5.96

120

3.8.96 Amplification of CHO D944 clones
CK/B1 and OAP from 3.4.96

CK/B1 (HWFB)68).
clones #s (for amplification)
3, 46, 48 growing before in 40 and 60 mM
all looked good
I split all 3 clones in MEM- + 5% diFBS, 50 mM,
100 mM, 200 mM, 500 mM in a 6 well dish
clones #s growing before in 50 mM. I split in MEM-
4, 24, 27 + 5% FBS + 60 mM MTX
OAP (HTPB322)
clones #s 4 and 6 growing in 60 mM confluent,
I split in MEM-, 50 mM and 100 mM (Riwell)

Obs: Results of the 535 labelling from 3.4.96
clones #s 1, 4, 6, 26 and 44 showed good production
#s 4 and 44 showed even better production
of OAP in 14 hours of exposure
On 3.8.96 I amplified the clones #s 4 and
6 in MEM- + 5% diFBS, 50 mM and 100 mM (Undil)

3/11/96 Amplification of CHO D944 clones from 3.8.96
CK/B1 (HWFB)68)
clone #s
48 growing before in 2- 50, 100, 200, 500 mM
split in 2-, 200, 300, 400 (100 of 50/100, 200)
the rest of 500 mM died

3 growing in α⁻, 50, 100, 200, 500 nM
split in α⁻, 200, 300, 500 nM (pool of 100, to 500)

#46 growing in α⁻, 50, 100, 200, 500 nM
split in α⁻, 200, 300, 500 (pool of 100, 200, 500)

#s 4, 24, 27 growing in 60 nM MTX & split in MEM α⁺ + 5% FBS in order to expand the cells fast for freezing as a back up.

In general the clones of CKBs looked good!

ODP (H+PBS22) from 3.8.96

Clones #s

3.9.96
4 growing in α⁻, 50, 100 nM
split in α⁻, 100, 200, 300 (pool of 50, 100 nM)
6 growing in α⁻, 50, 100 nM
split in α⁻, 100, 200, 300 (pool of 50, 100 nM)

1 growing in 60 nM MTX → in 24 well
26 " " "
44 " " "

9 split in α⁻, 100 nM #1
α⁻, 100, 200 nM #26 > 6 well
α⁻, 100, 200 nM #44
I did not use this concentration only 100 nM!

3.12.96 Harvested Spinner flask: infected on 5.3.96

2 spinners +6F2 → supernatant to [illegible]

6 " ESP-2 → " to Stephen

3.13.96 Amplification of CHO TNF clones HIV... from 3.6.96

Obs: on 3.11.96 I changed the media of all clones (MEM α+ 5% FBS) to recover the cells a lit bit because it was not so good. And on 3.13.96 the cells looked good [illegible] I started to split.

Clone #5

2 met the cells growing in 2 μM in 1, 2, 4, 6 μM

9 " " " " in 5 μM in 1, 5, 7, 10 μM

7 " " " " in 5 μM in 5, 7, 10 μM

6 " " " " in 6 μM in 6, 8, 10 μM

3 " " " " in 6 μM in 6, 8, 10 μM

2 " " " " in 2 μM in 1, 2, 4, 6 μM

2 " " " " in 5 μM in 1, 5, 7, 10 μM

[illegible signature]

3.B96 Injection Sf9 cells with plaque purified virus from 3.6.96

A2 HUPEN62

H10AU65 gp TNF R soluble

3.M96 Harvested transfection in Sf9 cells from 3.8.96

HSVA386.506 gp

IGF-1 mature peptide fused to gp signal peptide

3.M96 Freezing of CHO clones CKB-1 HWPBD68 from 3.M.96

the clones are resistant up to 60nM MTX

freezing media: MEMα+, 5% DMSO, 10% FBS

clones frozen #4, 24, 27

3 vials of each

Overnight at -70°C and after at -140°C

cell density/vial - 3x10⁶ cells/ml

[signature]

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☑ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.